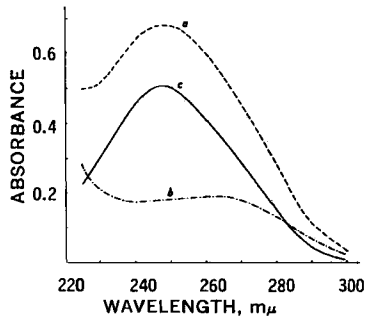

Fig. 5—Determination of prednisolone in tablets. See Fig. 1 for key.

presence of a 50-fold quantity of phenylbutazone having nearly the same absorbance at about 240 mμ as the prednisolone. In the presence of both active ingredients (alcohol extraction), the measurements, owing to the high background absorbances, could only be made in dilute solutions. The sensitivity and precision of the method was extremely decreased by this circumstance. To avoid this, an attempt was made to select the extraction. This could be achieved by the use of a 0.01 *N* solution of hydrochloric acid. In this solution prednisolone dissolves satisfactorily for the purpose given, while phenylbutazone being present at this acidity quantitatively in the keto form is dissolved only slightly. Thus the extraction of prednisolone becomes nearly selective. The small amount of phenylbutazone extracted has carbonyl groups of acid hydrazide type, which do not react with sodium borohydride, therefore the differential curve (Curve c, Fig. 5) is suitable for the correct estimation of prednisolone. The background (Curve b) is characteristic of the spectrum of phenylbutazone measured in alkaline solution [$\lambda_{max.}$ 264 mμ (9)].

REFERENCES

(1) Higuchi, T., and Brochmann-Hanssen, E., "Pharmaceutical Analysis," Interscience, New York, N. Y., 1961, p. 92.
(2) Kitazawa, T., Naito, M., and Yoshida, F., *J. Pharm. Soc. Japan*, **80**, 116(1960).
(3) Higuchi, T., and Brochmann-Hanssen, E., "Pharmaceutical Analysis," Interscience, New York, N. Y., 1961, pp. 88, 94.
(4) Garrat, D. C., "The Quantitative Analysis of Drugs," Chapman and Hall, London, England, 1964, pp. 585–600.
(5) Görög, S., *Steroids*, **11**, 93(1968).
(6) Neudert, W., and Röpke, H., "Atlas of Steroid Spectra," Springer Verlag, Berlin, Germany, 1965, No. 687.
(7) *Ibid.*, No. 726.
(8) Wheeler, O. H., and Kaplan, L. A., "Organic Electronic Spectral Data," vol. III., Wiley, New York, N. Y., 1966, p. 153.
(9) "British Pharmacopoeia," The Pharmaceutical Press, London, England, 1963, p. 604.

Keyphrases

Steroids—analysis
Ketosteroids, conjugated—determination in pharamaceuticals
Sodium borohydride reduction—steroid analysis
UV spectrophotometry—analysis

Effect of Physostigmine upon the Output of Catecholamines from the Adrenal Gland of the Rat

By C. L. KAUL and R. S. GREWAL

An investigation has been made on the effect of physostigmine on the output of catecholamines from the adrenal gland of the rat. Physostigmine (20 mcg. i.v.) causes three- to fourfold increase in the catecholamines output from the adrenal gland of the rat. This effect is mediated centrally as no increase was seen in the pithed animals. It is concluded that this peripheral release of catecholamines does not play any significant role in the hypertensive response of physostigmine in the rat.

INTRAVENOUS administration of physostigmine causes an appreciable rise in blood pressure in an urethan-anesthetized rat (1, 2). This pressor response of physostigmine has been mainly attributed to central adrenergic stimulation and is absent or much less in pithed animals (2). Although the hypertensive response of physostigmine is mainly central, there is some evidence to suggest that some peripheral action may also be involved (2–4). Medaković and Varagić (5) have also postulated that liberation of epinephrine and norepinephrine from the adrenals does not seem to play a significant role in the hypertensive response to physostigmine, as the response to physostigmine was the same in normal or adrenalectomized animals.

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Physostigmine is known to increase the output of epinephrine (10–15-fold) from the cat adrenals (6). According to these authors, this effect is mediated centrally, as no increase in the output of epinephrine was demonstrated when the splanchnic and other nerve supply to the adrenal glands was cut. Since physostigmine is known to cause central adrenergic stimulation in the rat, it is possible that this effect is conveyed to the periphery through established sympathetic pathways and one would expect it to cause a release of catecholamines from the rat adrenals (as has been shown in the case of cats). This release of catecholamines could possibly contribute to the hypertensive effect of physostigmine.

The purpose of the present work was to see if physostigmine increased the output of catecholamine from the adrenal gland in the rat and if this output contributes to its hypertensive response. Therefore it was also thought desirable to compare the pressor response of epinephrine, norepinephrine, and physostigmine in normal and adrenalectomized animals to see if there are any differences in the response of the cardiovascular system under these conditions. Finally the effect of β-blocker on the hypertensive response of physostigmine in normal and adrenalectomized rats was also investigated.

METHODS

Adult male rats (C.F. strain) weighing from 300–340 g. (Series 1–3) and 250 g. (Series 4) were used. They were anesthetized with urethan (1.5 g./kg.). Four series of experiments were carried out in these animals. In the first series the pressor response to 20 mcg. of physostigmine was measured in normal and adrenalectomized animals. In the second series pressor response to intravenous injection of epinephrine and norepinephrine was measured in normal and adrenalectomized rats. In both these sets of experiments the blood pressure was recorded from a common carotid artery by a mercury manometer writing on a smoked kymograph. In the third series of experiments the total catecholamines were measured as norepinephrine from the venous effluent of the main adrenal vein (side branch tied) during control period and after intravenous injection of 20 mcg. of physostigmine. In these experiments both the jugular veins were cannulated, one for injection of the drugs and the other for giving continuous infusion of heparinized saline (0.1 ml./min.) till the end of the experiment. Blood 0.5–0.7 ml., was collected in about 30 min. from a cannulated adrenal vein in a cold centrifuge tube, containing heparin. The plasma was separated by centrifugation (about 2,000 r.p.m.) and it was assayed against norepinephrine on the blood pressure of a pithed rat. As the volume of the plasma was very small, the unknown plasma sample was bracketed between two or three doses of standard norepinephrine. In some experiments where the volume of plasma was higher, two lines of a Latin square were carried out for assay. Three samples were usually collected from each rat, one at 0 hr. before giving physostigmine and the other two at 30 and 60 min. after giving physostigmine. Control experiments were carried out without physostigmine and the samples were collected at 0, 30, and 60 min. and were assayed against norepinephrine to see if there were any increase in the amount of catecholamines released during this time.

To confirm whether the effect of physostigmine was central or peripheral, a set of experiments (Series 4) was done using pithed animals and in this set also three samples of blood were collected and assayed as described above. In these experiments the blood flow was very low and a longer period of collection (40 min.) was necessary to collect approximately the same volume of blood so that an assay could be carried out and the rate of heparinized saline infusion had also to be increased (0.2–0.3 ml./min.).

For the estimation of total catecholamines from the adrenal gland, extraction of the tissues was carried out by perchloric acid and the extract after centrifugation was assayed on the spinal cat (blood pressure) using epinephrine as a standard.

Drugs used—Epinephrine acid tartrate; *l*-norepinephrine-*d*-hydrogen tartrate; physostigmine salicylate; and propranolol.

All concentration and doses of these drugs refer to their salts except for epinephrine and norepinephrine where it is expressed as a free base. Doses of physostigmine are expressed per rat.

RESULTS

Results of the first group of experiments show that pressor responses to 20 mcg. of physostigmine in adrenalectomized animals is slightly but significantly higher than the controls (Table I). There was however no significant difference in the pressor responses of epinephrine or norepinephrine in normal and adrenalectomized animals at different time intervals (Table II).

The effect of physostigmine on the catecholamine output is shown in Table III. From this table it can be seen that there is a 3–4-fold increase in the amount of catecholamine output measured as norepinephrine. This increase in the catecholamine output was seen 0.5 hr. after intravenous injection of physostigmine and persisted up to 1 hr. when the last sample of blood was collected. In a set of control experiments where no physostigmine was given, there was some increase in the amount of catecholamine output in the blood collected at 1 hr. However, the difference between the two means was not statistically significant. In pithed animals, the control levels of catecholamines were lower than the normal animals and physostigmine produced no significant increase in the output of

TABLE I—BLOOD PRESSURE RESPONSE (mm. Hg) TO PHYSOSTIGMINE (20 mcg.) IN NORMAL AND ADRENALECTOMIZED RATS[a]

Treatment	B.P. Rise, mm. Hg
Normal	54.20 ± 3.19 (14)[b]
Adrenalectomized	66.3 ± 2.73 (10)[c]

[a] All values are the means ± SEM. [b] Figures in the brackets indicate the number of observations. [c] $p < 0.01$.

TABLE II—BLOOD PRESSURE RESPONSES (mm. Hg) TO EPINEPHRINE (0.5 mcg.) AND NOREPINEPHRINE (0.5 mcg.) IN NORMAL AND ADRENALECTOMIZED RATS[a]

Normal					
0 hr.		0.5 hr.		1 hr.	
Epinephrine	Norepinephrine	Epinephrine	Norepinephrine	Epinephrine	Norepinephrine
Normal					
39.30 ± 4.47	42.60 ± 5.77	39.10 ± 4.99	34.00 ± 4.49	40.10 ± 6.76	31.60 ± 4.54
Adrenalectomized					
52.60 ± 4.22	43.30 ± 8.92	54.00 ± 7.44	35.30 ± 8.08	40.30 ± 5.09	31.30 ± 5.54

[a] All values are the means ± SEM of determinations on 6 rats.

TABLE III—CATECHOLAMINE OUTPUT FROM THE ADRENAL VEIN IN CONTROL RATS AND RATS GIVEN PHYSOSTIGMINE[a]

Treatment	Drug	Dose, mcg.	Concn., mcg./ml., Calcd. as Norepinephrine		
			0 hr.	30 min.	60 min.
Control	—	—	0.16 ± 0.03 (8)[b]	0.22 ± 0.04 (9)	0.26 ± 0.05 (9)
Normal rats	Physostigmine	20	0.20 ± 0.02 (15)	0.65 ± 0.11 (12)[c]	0.86 ± 0.18 (11)[c]
Pithed rats	Physostigmine	20	0.023 ± 0.002 (6)	0.027 ± 0.006 (6)	0.021 ± 0.006 (6)

[a] All values are the means ± SEM. [b] Figures in the brackets indicate the number of observations. [c] $p < 0.001$.

catecholamines (Table III). Since the catecholamine concentration was lower in the plasma of the pithed animals, it was also of interest to see if pithing itself produced any significant effect on the catecholamine content of the rat adrenals. The results of these experiments are shown in Table IV. From this table it can be seen that there is a significant reduction in the catecholamine content of adrenal gland after pithing.

The experiments reported with propranolol (Table V) show that in both normal and adrenalectomized animals, propranolol potentiated the hypertensive response of physostigmine slightly. In adrenalectomized animals, with the higher dose of propranolol the increase in the pressor response was significantly higher than the normal rats (65%).

TABLE IV—THE EFFECT OF PITHING[a] ON THE CATECHOLAMINE CONTENT OF RAT ADRENALS[b]

Treatment	Concn., mcg./g.
Control	931.0 ± 40.4 (21)[c]
Pithed	676.0 ± 30.0 (11)[d]

[a] The animals were pithed under light ether anesthesia and were kept on artificial respiration for 0.5 hr. before the adrenals were taken out. [b] All values are the means ± SEM. [c] Figures in the brackets indicate the number of observations. Six adrenals from 3 rats were used for each observation. [d] $p < 0.001$.

Varagić *et al.* (7) have also reported slight increase in the pressor response of physostigmine in rats given propranolol (21.7 mg./kg.).

DISCUSSION

The results presented in this paper show that intravenous administration of physostigmine in normal rats increases the output of catecholamines from the adrenals. This increase in the output of catecholamines was absent in pithed animals, indicating thereby that physostigmine produces the effect centrally and no peripheral action is involved. Similar results have been reported by Stewart and Rogoff (6) in cats.

The lower concentration of catecholamines found in the plasma of pithed animals is probably related to the fact that a significant reduction in the total catecholamine content of the adrenal gland occurs after pithing. It perhaps also indicates that central adrenergic mechanisms have a certain tonic influence on the release of adrenal medullary hormones and in the absence of this central influence, as happens in pithing, the amount of hormone release is less. Another contributory factor would be reduction in the blood supply to the adrenals consequent to the lowering of blood pressure after pithing. Harvey *et al.* (8) have also reported low values of plasma catecholamines in pithed dog

TABLE V—EFFECT OF PROPRANOLOL (100 AND 200 mcg./kg.) ON THE HYPERTENSIVE RESPONSE OF PHYSOSTIGMINE (20 mcg.) IN NORMAL AND ADRENALECTOMIZED RATS[a]

Treatment	Dose, mcg./kg.	B.P. Response, mm. Hg	
		Before Propranolol	After Propranolol
Normal	100	55.61 ± 5.09 (6)[b]	71.10 ± 10.98 (6)
Normal	200	54.30 ± 6.10 (6)	65.20 ± 3.70 (5)
Adrenalectomized	100	59.10 ± 5.60 (6)	76.30 ± 5.96 (6)
Adrenalectomized	200	52.60 ± 6.90 (6)	87.30 ± 9.70 (6)[c]

[a] All values are the mean ± SEM. [b] Figures in the brackets indicate the number of observations. [c] $p < 0.05$.

and these low values have been partly attributed by these authors to the pithing of the animals.

The peripheral release of catecholamines (epinephrine and norepinephrine) does not seem to play any significant role in the hypertensive response of physostigmine (5, 9). This observation is based on the fact that these authors did not find any difference in the pressor response of physostigmine in normal as well as in the adrenalectomized animals. They further suggested (9) that although physostigmine is known to increase the output of epinephrine from the adrenals of the cat (6) it is unlikely that this effect occurs in the rat. These results contradict their statement that physostigmine will not increase the peripheral release of catecholamines from the adrenals of the rat. The results presented in this paper clearly demonstrate such an effect in the rat.

The authors have shown that catecholamines are being released following injection of physostigmine and if these were contributing to the hypertensive response then one would have seen a bigger rise of blood pressure following physostigmine injection in normal animal with intact adrenals than in adrenalectomized animals. In fact the experiments showed an increase in the pressor response of physostigmine in adrenalectomized animals. Therefore these results show that release of catecholamines from the adrenal glands does not contribute to the hypertensive response of physostigmine.

Some doubt also exists in the literature as to whether the adrenal medullary secretion plays any significant role in the cardiovascular regulations (10). Since most of the catecholamine present in the adrenal medulla is adrenaline (85%) (11), it is possible that its effect on β-adrenergic receptors in certain vascular beds leads to vasodilation and masks the vasoconstriction effect. This could probably explain why a significant increase in the pressor response of physostigmine in the adrenalectomized animals as compared to controls is seen. This could not be attributed to the change of sensitivity of the cardiovascular system, after adrenalectomy, since pressor responses of epinephrine and norepinephrine were the same in both normal and adrenalectomized animals. This is further supported by experiment with propranolol where an increase in the pressor response of physostigmine occurred. The slight but significant ($p < 0.05$) increase in the pressor response in the adrenalectomized animals is difficult to explain with the data available.

REFERENCES

(1) Dirnhuber, P., and Cullumbine, H., *Brit. J. Pharmacol.*, **10**, 12(1955).
(2) Varagić, V., *ibid.*, **10**, 349(1955).
(3) Burn, J. H., and Weetman, D. F., *ibid.*, **20**, 74(1963).
(4) Della Bella, D., Gandini, A., and Preti, M., *ibid.*, **23**, 540(1964).
(5) Medaković, M., and Varagić, V., *ibid.*, **12**, 24(1957).
(6) Stewart, G. N., and Rogoff, J. M., *J. Pharmacol. Exptl. Therap.*, **17**, 227(1921).
(7) Varagić, V. M., Terzić, M., and Mršulja, B. B., *Arch. Exptl. Pathol. Pharmakol.*, **258**, 229(1967).
(8) Harvey, S. C., Sulkowski, T. S., and Weening, D. J., *Arch. Intern. Pharmacodyn.*, **169**, 262(1967).
(9) Lesić, R., and Varagić, V., *Brit. J. Pharmacol.*, **16**, 99(1961).
(10) Celander, O., *Acta Physiol. Scand. Suppl.*, **32**, 116 (1954); cited by Uvnäs, B. in "Neurophysiology," Vol. II, Field, J., Magon, H. W., and Hall, V. E., Eds., American Physiological Society, Washington, D. C., 1960 p. 1158.
(11) Hökfelt, B., *Acta Physiol. Scand. Suppl.*, **25**, 92(1951).

Keyphrases

Physostigmine—catecholamine output
Adrenal gland catecholamine output—physostigmine effect
Central mediation—physostigmine activity

In Vitro Utilization of Diethylstilbestrol by Rat Liver

By MARÍA GABALDÓN, JUAN SÁNCHEZ, and ANTONIO LLOMBART, JR.

A technique is described for the study of the utilization of diethylstilbestrol (DES) by rat liver *in vitro*. The disappearance of DES and the seasonal variations were determined on liver slices and homogenates. A chromatographic identification of diethylstilbestrol monoglucuronide (DESGA) as a reaction product in the assays of slices was carried out. The effect of liver preincubation with saline phosphate buffer, of phenylmercuric acetate, and of uridine diphosphate glucuronic acid on DES utilization by liver was studied. The possibility of a DES utilization pathway other than monoglucuronide formation is discussed. A thin-layer chromatographic study was carried out with DES and DESGA.

DIETHYLSTILBESTROL (α,α' - diethylstilbenediol) is an orally active synthetic estrogen that has been utilized in therapeutics to produce prostatic tumor regression; it inhibits glutamate dehydrogenase by breaking the enzyme into inactive subunits (1), uncouples oxidative phosphorylation with mitochondrial volume expansion (2), and inhibits electron transport between cytochromes *b* and *c* (3).

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